

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/20	A1	(11) International Publication Number: WO 99/56741 (43) International Publication Date: 11 November 1999 (11.11.99)
(21) International Application Number: PCT/US99/09505 (22) International Filing Date: 30 April 1999 (30.04.99) (30) Priority Data: 60/083,965 1 May 1998 (01.05.98) US 60/099,431 8 September 1998 (08.09.98) US Not furnished 28 April 1999 (28.04.99) US (71)(72) Applicant and Inventor: SEE, Jackie, R. [US/US]; 541 Riviera Court, Fullerton, CA 92835 (US). (74) Agent: OLSTER, Kathleen, M.; Christie, Parker & Hale, LLP, P.O. Box 7068, Pasadena, CA 91109-7068 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHODS FOR TREATING FEMALE SEXUAL DYSFUNCTION		
(57) Abstract		
<p>A method for treating female sexual dysfunction or for female sexual enhancement comprises administering to the vagina a composition comprising a prostanoid compound having a vasodilating effect in a pharmaceutically acceptable vehicle. The amount of prostanoid compound administered ranges from about 0.05 mg to about 3 mg, and preferably from about 0.125 mg to about 1 mg. Preferably the prostanoid compound is prostaglandin E-1 and the pharmaceutically acceptable vehicle is methylcellulose.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHODS FOR TREATING FEMALE SEXUAL DYSFUNCTION

Field of the Invention

This invention relates to the methods for treating female sexual dysfunction and for female sexual enhancement, and more particularly to the administration of a prostanoid compound to a female intravaginally or extravaginally.

Background of the Invention

Recent studies have revealed that approximately 10 million women in the United States between the ages of 50 and 74 reported a lack of lubrication on 229 million sexual intercourse occasions. In fact, it was found that over fifty-eight percent of 260 females surveyed were affected by sexual dysfunction, as defined as pain or discomfort during sexual intercourse, diminished vaginal lubrication, delayed vaginal engorgement, increased time for arousal, diminished ability to reach orgasm, or diminished clitoral sensation.

An animal model of this syndrome was developed by Goldstein and Berman (Int. J. Impot. Res. 10 Suppl. 2:S84-90; S98-101, 1998, the disclosure of which is incorporated herein by reference). These investigators undertook diagnostic studies assessing the hemodynamic integrity of the ilio-hypogastric-pudendal arterial bed to the vagina and clitoris and new oral/topical pharmacologic strategies for enhancing vaginal/clitoral blood flow in patients with vasculogenic female sexual dysfunction. They observed that there was a growing body of evidence that women with sexual dysfunction would commonly have physiologic abnormalities, such as vasculogenic female sexual dysfunction, contributing to their overall sexual health problems.

Park et al. (Int. J. Impot. Res. 9:27-37, 1997, the disclosure of which is incorporated herein by reference) have shown that female sexual dysfunction may be related in part to vasulogenic impairment of the hypogastric-vaginal/clitoral arterial bed. In order to investigate this theory under a controlled experimental situation, they developed an animal model of vaginal engorgement insufficiency and clitoral erectile insufficiency. They achieved pelvic nerve stimulated vaginal engorgement and clitoral erection in control normal diet New Zealand White female rabbits and atherosclerotic balloon injury of aorto-iliac arteries and 0.5% cholesterol diet New Zealand White female rabbits. After 16 weeks, novel hemodynamic variables, including vaginal wall and clitoral blood flow, vaginal wall and clitoral intracavernosal pressure, vaginal length, vaginal luminal pressure, blood levels of cholesterol and triglycerides, aorto-iliac angiography and vaginal wall and clitoral erectile tissue histology, were recorded in the two groups of rabbits. Their results indicate that pelvic nerve stimulation induced vaginal hemodynamic changes, and there was significantly less increase in blood flow, wall pressure and length changes in atherosclerotic rabbits compared to the control rabbits. Histologic examination of clitoral erectile tissue demonstrated

1 cavernosal artery atherosclerotic changes and diffuse vaginal and clitoral fibrosis. Aorto-iliac
angiography in atherosclerotic animals revealed diffuse moderate to severe atherosclerotic
occlusion. They conclude that vaginal engorgement and clitoral erection depend increased blood
5 inflow.

Present day management of women with sexual arousal disorder, especially those with
diminished vaginal lubrication and painful penetration is based on psychologic, hormonal and
"artificial lubricant" interventions. The pathophysiology of vaginal engorgement insufficient may
be, in part, related to vascular impairment.

10 Prostanoid compounds, such as prostaglandin E-1 (PGE-1), have been used as an adjunct
to improving blood flow in arterial angioplasty procedures. See See et al., Advances in
Prostaglandin, Thromboxane and Leukotriene Res., 17: 266-70, 1987; See et al., Applied
Cardiopulmonary Pathophysiology 2:193-98, 1998, the disclosures of which are incorporated
herein by reference. Prostanoid compounds have also been used in the treatment of male erectile
15 dysfunction. For example, U.S. Patent No. 5,718,917, the disclosure of which is incorporated
herein by reference, discloses a method for treating erectile dysfunction comprising administering
prostaglandin-containing liposomes to the penis.

Summary of the Invention

20 The present invention provides methods for treating female sexual dysfunction and for
female sexual enhancement. The invention is also directed to methods for enhancing blood flow
in the vagina of a patient. The methods comprise administering a composition comprising a
prostanoid compound having a vasodilating effect in a pharmaceutically acceptable vehicle to the
vagina.

25 A preferred dose of the composition contain less than 3 mg of the prostanoid compound,
more preferably from 0.05 mg to about 3 mg, more preferably 0.05 mg to about 2 mg, and still
more preferably about 0.125 mg to about 1 mg of the prostanoid compound. In a preferred
embodiment, the prostanoid compound is contained within liposomes, which preferably are
lyophilized to enhance the stability of the compositions. In a preferred embodiment, the
30 prostanoid composition is lyophilized. Preferably the prostaglandin is prostaglandin E-1 ("PGE-
1").

Description of the Drawings

35 These and other features and advantages of the present invention will be better understood
by reference to the following detailed description when considered in conjunction with the
accompanying drawings wherein:

1
FIGs. 1 to 4 are tables showing the graphic distribution of PGE-1 concentration over the duration of a stability study.

5
FIG. 5 is a table showing the relative blood flow in the vaginal muscle compared to the leg muscle upon administration of a PGE-1 composition to the vagina.

Detailed Description

10
The present invention is directed to methods for treating female sexual dysfunction and for female sexual enhancement. As used herein, female sexual dysfunction includes pain or discomfort during sexual intercourse, diminished vaginal lubrication, delayed vaginal engorgement, increased time for arousal, diminished ability to reach orgasm, and diminished clitoral sensation. The invention is also directed to methods for enhancing blood flow in the vagina of a patient. The methods comprise administering a composition comprising a prostanoid compound having a vasodilating effect in a pharmaceutically acceptable vehicle to the vagina.
15 externally and/or internally.

As used herein, the term "vagina" refers not only to the genital canal that extends from the uterus to the vulva, but also to the external genitalia, including the clitoris, labium anterium, labium posterius, labia majora, and labia minora. More specifically, the clitoris contains erectile tissue, blood supply and innervation in the female similar to that in the penis of the male. It has
20 been discovered that the application of the composition to the clitoris increases the blood supply to engorge the clitoris by the same mechanism as engorgement of vascular tissue of the penis in the male. The composition's effects on the innervation of the erectile tissue and sensory tissue of the clitoris in the female is similar to its effects on the comparable male tissue.

Prostanoid compounds suitable for use in the present invention include prostaglandin E
25 (PGE), including PGE-1, PGE-2 and PGE-3, prostaglandin A (PGA), including PGA-1, prostaglandin F (PGF), including PGF-2, prostaglandin D (PGD), including PGD-2, prostacylins, thromboxanes, leukotrienes, 6-keto-PGE-1 derivatives, carbacyclin derivatives, PGD-2 derivatives and the like, and synthetic analogs of any prostanoid compounds. PGE-1 and PGE-2 are preferred, and PGE-1 is the most preferred. Other examples of suitable prostanoid compounds
30 are described in U.S. Patent No. 4,955,878, the disclosure of which is incorporated herein by reference. The prostanoid compound is preferably provided in crystal, liquid or lyophilized form within the vehicle.

A preferred dose contain less than 3 mg of the prostanoid compound, more preferably from 0.05 mg to about 3 mg, more preferably 0.05 mg to about 2 mg, and still more preferably about
35 0.125 mg to about 1 mg of the prostanoid compound. The dose of prostanoid compound should be maintained at these relatively low levels due to the potential toxicity of the prostanoid compound caused by rapid absorption of the prostanoid compound across the vaginal tissue.

1 The pharmaceutically acceptable vehicle is a composition to facilitate the application of the
prostanoid compound. Suitable vehicles include, for example, lotions, gels and liquids. A
particularly preferred vehicle is methylcellulose, such as that sold under the name KY JELLY.
5 Preferably the vehicle has a near-neutral pH. Other examples of vehicles include water, silicone,
waxes, polyethylene glycol, propylene glycol and sugars. The compositions are formed by
uniformly mixing the prostanoid compound in the vehicle according to methods known to those
skilled in the art.

10 In a preferred embodiment, the prostanoid compound is contained within liposomes. The
liposomes of the present invention may be made of any suitable phospholipid, glycolipid, derived
lipid, and the like. Examples of suitable phospholipids include phosphatide choline, phosphatidyl
serine, phosphatidic acid, phosphatidyl glycerin, phosphatidyl ethanolamine, phosphatidyl inositol,
sphingomyelin, dicetyl phosphate, lysophosphatidyl choline and mixtures thereof, such as soybean
15 phospholipids, and egg yolk phospholipids. Suitable glycolipids include cerebroside,
sulphur-containing lipids, ganglioside and the like. Suitable derived lipids include cholic acid,
deoxycholic acid, and the like. The presently preferred lipid for forming the liposomes is egg
phosphatidylcholine.

20 The liposomes may be formed by any of the known methods for forming liposomes and
may be loaded with prostaglandin according to known procedures. Known methods for forming
liposomal prostaglandin (PG) are described, for example, in PCT Application No.
PCT/US88/01714 and European Patent Application No. EP 0,512,916A2, both assigned to the
Liposome Company, and European Patent Application No. EP 0,416,527A2 assigned to the
Green Cross Corporation, and the references disclosed in those applications, all of which are
incorporated herein by reference. What is formed is an emulsion comprising liposomal PG.

25 It is preferred that the liposomes used in the present invention have an average mean
diameter from about 20 nm to about 1000 nm and preferably of from about 100 nm to about 200
nm. An average mean diameter of about 140 nm is particularly preferred. Liposomes larger than
about 1000 nm are not preferred because they are difficult to make. Liposomes smaller than
about 20 nm are usable but not preferred because they are difficult to make.

30 Accordingly, the liposomes produced are preferably treated to reduce their size and to
produce a homogeneous population. This may be accomplished by conventional techniques such
as extrusion through a filter preferably of 100 to 500 nm pore size, the filter being either the
straight path or tortuous path type. Other methods of size reducing the liposomes to a
homogenous size distribution are ultrasonic exposure, the French press technique, hydrodynamic
35 shearing, homogenization using, for example, a colloid mill or Gaulin homogenizer or
microfluidization techniques. Microfluidization is presently preferred, and is described in U.S.
Patent No. 4,533,254 to Cook, et al., the disclosure of which is incorporated herein by reference.

1 In a preferred microfluidization procedure, the liposomal emulsion is forced at high pressure through a small diameter opening, splattered onto a wall and then collected.

5 In a particularly preferred embodiment of the invention, the liposomes are passed one to ten, and preferably four, times through an M-110 Series Laboratory Microfluidizer manufactured by Microfluidics Corporation at a pressure of, e.g., 14000-16000 pounds per square inch to achieve a generally homogenous population of liposomes having an average mean diameter of about 140 nm.

10 Lyophilization of the prostanoid compound or liposomal prostanoid compound may be accomplished by any method known in the art. Such procedures are disclosed, for example, in U.S. Patent No. 4,880,836 to Janoff, et al., the disclosure of which is incorporated herein by reference. In the case of liposomes, lyophilization procedures preferably include the addition of a drying protectant to the liposome suspension. The drying protectant stabilizes the liposomes so that the size and content are maintained during the drying procedure and through rehydration. Preferred drying agents are saccharide sugars including dextrose, sucrose, maltose, manose, galactose, raffinose, trehalose lactose, and triose sugars which are preferably added in amounts of about 5% to about 20% and preferably about 10% by weight of the aqueous phase of the liposomal suspension. Dextrose, sucrose and maltose are presently preferred. Manitol may be used in conjunction with any of the saccharides. Additional preservatives such as BHT or EDTA, urea, albumin, dextran or polyvinyl alcohol may also be used.

20 In the case of liposomal PG, to determine the amount of liposomal PG necessary to deliver the desired dose of prostaglandin, it was necessary to determine how much prostaglandin is present. Analysis using a standard prostaglandin E-2 ELISA test established that prostaglandin can be loaded into liposomes in amounts as high as 20% to 40% by weight. However, it is preferred that the prostaglandin be present in the liposomes in an amount of from about 2% to about 3% by weight. A particularly preferred lyophilized liposomal PG composition comprises 44 mg egg phosphatidylcholine, 75 mg maltose and 1 mg prostaglandin E-1.

25 If desired, the composition can further comprise a surfactant to enhance the bioavailability of the prostanoid compound. The surfactant reduces the interfacial tension between the pharmaceutically acceptable vehicle and the prostanoid compound, and also reduces the interfacial tension between the prostanoid compound and the vaginal mucosa. When the prostanoid compound is encapsulated in liposomes, a surfactant can also enhance the dissolution of the liposomes. Suitable surfactant formulations for use in the present invention are described in U.S. Patent No. 5,207,220, the disclosure of which is incorporated herein by reference. Preferably the surfactant is present in the composition in an amount ranging from about 0.02% to about 2% by weight.

Other agents can be included in the composition of the invention. including, but not limited to, other vasodilators, alpha-adrenergic inhibitors, prazosin, papaverine, phentolamine, vasointestinal peptide (VIP), estrogen and estrogen-like compounds, alpha-adrenergic receptor mediators, nitroglycerin compounds and the like.

Example 1

Preparation of Lyophilized PGE-1 Liposomes

Liposomal PGE-1 in accordance with the present invention was prepared according to the following procedure.

1. 2250 ml of water (double distilled) to beaker (keep cool) and set with a nitrogen sparge for at least 30 minutes.

2. Add 225 gms of maltose (Sigma M5885) to the water and mix until dissolved. Keep the nitrogen sparge going. Mixture at pH of 4.81.

3. In another beaker 10.59 gms of egg phosphatidylcholine (EPC) (Sigma) is combined with 8.38 ml of ethanol (anhydrous, Sigma E3884) and mixed until dissolved. To this add 67.5 mg of BHT and mix until dissolved. To this mixture add 2160 mg of PGE-1 and mix until dissolved. Use the remaining 4.19 ml of ethanol to rinse any remaining PGE-1 in the weighing container into the mixture.

4. Draw the ethanol solution into a 10 ml glass syringe and add to the maltose solution over 11 minutes with continued nitrogen sparge. Keep pH <7.0 (goes into microfluidizer at pH 4.81). Measure. Hand blend mixture. Keep everything cool 1.5 degrees C.

5. Microfluidizer. Four (4) passes through the microfluidizer 110F: 9,000 Units

	<u>Total Weight of Materials</u> <u>to be Used*</u>	<u>Based on Previous Run</u> <u>(886 Units)*</u>
EPC	10.59 grams	107.573 grams
Maltose	225 grams	2,285.55 grams
Ethanol	12.57 ml	127.69 ml
BHT	67.5 mg	685.66 mg
PGE-1	2160 mg	21,941 grams
(USP)Water	2250 ml	22,855.5 ml

Pressure 16,000 PSI

Caution-keep acidic, keep temp (melting point 115 degrees)

Note-Maltose melting point 102-103 degrees Centigrade

1 * Multiplier + 10.158

5 6. Take 2.7 ml of the finished product and lyophilize in approximately 1,000 6 ml
Wheaton eye dropper bottles.

Lyophilization was accomplished according to the following cycle:

1. Shelf at $\leq -45^{\circ}\text{C}$ for at least one (1) hour before loading.
2. Load product and keep at $\leq -45^{\circ}\text{C}$ for twelve (12) hours.
3. Vacuum to $\sim 50\mu$.
- 10 4. Shelf temperature maintained at -28°C to -20°C for 59 hours.
5. Shelf temperature rose from -20°C to -5°C during subsequent ten (10) hours.

Visually product needed extra time at -20°C .

6. Shelf reset at -22°C and maintained at -22°C to -18°C for thirty-six (36) hours.
7. Shelf reset $+25^{\circ}\text{C}$ and held at 25°C for 48 hours.

15 It is anticipated that the following lyophilization cycle will provide the same results in a
shorter time.

1. Shelf to $\leq -45^{\circ}\text{C}$ for at least one (1) hour before loading.
2. Load product, keep at $\leq -45^{\circ}\text{C}$ for at least six (6) hours.
3. Vacuum to $\leq 100 \mu$.
- 20 4. Shelf to -28°C for 50 hours.
5. Shelf to $+25^{\circ}\text{C}$ for 40-50 hours.

Example 2

Vaginal Application of PGE-1 in KY Jelly on Rabbits

25 Treatment lotion was prepared as follows. Liposomal PGE-1, made in a manner similar
to that described above, was prepared. Six sets of treatment lotion having different
concentrations of PGE-1 were prepared by combining 1 gm of KY Jelly (methylcellulose,
available from Ortho Pharmaceutical Corporation) with liposomal PGE-1 to achieve a final
30 amount of PGE-1 equal to 1.5, 1.0, 0.5, 0.25, and 0.125 mg, and without liposomal PGE-1 as a
control. The treatment lotion was kept refrigerated until used.

Thirty ten-week old female rabbits were kept in cages and fed, watered and observed daily.
The treatment lotion was applied daily to the rabbits over a period of fourteen days, and the
rabbits were observed daily. The rabbits were sacrificed 1+ hours after the final dose was applied.

35 Each rabbit was given 100 mg of Xylazine and 100 mg of ketamine intramuscularly using
a 3 cc syringe and a 22 gauge needle. Once the rabbit was under anesthesia, the chest and groin
area was clipped, and the animal was brought into the surgical area. Blood was drawn by heart

1 puncture and transferred to a 7 cc heparin tube. The chest was then opened, and the heart was
removed to insure a quick and painless death. Three sections were taken from the vaginal area
and placed in 2 cc of formalin. After the surgeries were complete, the blood was centrifuged for
5 10 minutes, and the plasma was frozen. The vaginal sections were fixed for at least 24 hours and
were then stained and fixed to slides.

In the rabbits treated with the PGE-1 containing compositions, increased vascular
engorgement, vasodilation and hyperemia were observed compared to the rabbits treated with
controls. Moreover, no systemic effects were observed, as indicated by a lack of degradation
10 products.

Example 3

Reconstitution Study for Topical Liposomal PGE-1

15 A 1.0 mg aliquot of lyophilized liposomal PGE-1 was mixed with 1.0, 2.0, 4.0 and 8.0 g
aliquots of KY Jelly to determine the stability of PGE-1 in the presence of KY Jelly. Each
preparation was mixed thoroughly by vortexing. The resulting mixtures contained 1.0 mg/ml, 0.5
mg/ml, 0.25 mg/ml and 0.125 mg/ml PGE-1, respectively. A 0.5 g aliquot of each preparation
was transferred to an Eppendorf centrifuge tube along with a 0.5 ml aliquot of acetonitrile. Each
preparation was extracted by vortexing thoroughly. After extraction, each preparation was
20 centrifuged, and the liquid supernatant was transferred to an autosampler vial for analysis.

In addition, 1.0 mg/ml and 0.5 mg/ml preparations were extracted using 2 ml and 4 ml
aliquots of acetonitrile for comparison. The different preparation procedures were utilized to
determine the best extraction process that would product the most homogeneous mixture for the
accurate identification and quantitation of the active ingredient, PGE-1.

25 All test preparations were extracted and analyzed by HPLC at 15, 30, 60 and 120 minute
intervals and at 1, 2 and 5 days. The results were evaluated for the accurate identification and
quantitation of PGE-1 and for any trends in loss of concentration over time.

Tables 1 to 4, below, show the individual assay results obtained for each preparation, and
Figures 1 to 4 provide a graphic distribution of PGE-1 concentration over the duration of the
study. The results indicate that the 1.0 mg/ml lyophilized liposomal PGE-1 topical product is
30 stable in KY Jelly for at least 2 days and does not degrade to metabolites.

Table 1 - Stability of PGE in K.Y. Jelly (1000 ug/ml PGE)

Time	Prep1	Prep2	Prep3	Average (ug/ml)
15 min	946	969.8	1011.5	975.8
30 min	997.8	1027	1073.7	1032.8
60 min	1109.8	942.8	1116.9	1056.5
120 min	987.1	941.7	945.4	958.1
1 day	882.5	843.5	861.9	862.6
2 days	942.6	920.1	898.6	920.4
5 days	738.8	751.2	685.1	725.1

Table 2 - Stability of PGE in K.Y. Jelly (500 ug/ml PGE)

Time	Prep1	Prep2	Prep3	Average (ug/ml)
15 min	471.3	297	518.3	428.9
30 min	549.6	434	486.6	490.1
60 min	450	536.9	581.5	522.8
120 min	504.4	484.5	463	484
1 day	458.1	441.6	390.7	430.1
2 days	497.9	507.4	501.3	502.2
5 days	281.8	303.6	294.9	293.4

Table 3 - Stability of PGE in K.Y. Jelly (250 ug/ml PGE)

Time	Prep1	Prep2	Prep3	Average (ug/ml)
15 min	222.4	221.5	203.2	215.7
30 min	247.2	233	301.7	260.6
60 min	149.3	190.4	143.4	161
120 min	222.6	378.2	380.8	327.2
1 day	213.9	218.5	226	219.5
2 days	179.3	227.9	188.8	198.7
5 days	186.3	144.9	171.2	167.5

Table 4 - Stability of PGE in K.Y. Jelly (125 ug/ml PGE)

Time	Prep1	Prep2	Prep3	Average (ug/ml)
15 min	101.3	114.6	103.5	106.5
30 min	105.2	108.8	99.1	104.4
60 min	106.3	102.3	91.9	100.2
120 min	108.3	101.4	96	101.9
1 day	111.3	111.6	118.3	113.7
2 days	97.8	103.1	88.4	96.4
5 days	87.4	84.9	79.1	83.8

Example 4

PGE-1 was encapsulated in multilamellar liposomes in a manner similar to that described in Example 1, above. Three different preparations were made: a control (1 gram of KY Jelly without any liposomal PGE-1), a low dose preparation (0.25 mg of liposomal PGE-1 uniformly mixed with 1 gm of KY Jelly), and a high dose preparation (2.0 mg of liposomal PGE-1 uniformly mixed with 1 gm of KY Jelly).

Female New Zealand rabbits were used as the animal model. MRI studies were initiated when the rabbits were 5 weeks old as the baseline study where the control preparation (C) was applied to the vagina. At that time, the average weight of the rabbits was 1510 ± 486 gm. A second study was performed a week later when the rabbits were 6 weeks old using the low dose preparation (LD). The average weight of the rabbits during the second study was 1746 ± 444 gm. A third study was performed 2 weeks after the baseline study when the animals were 7 weeks old

1 where the high dose preparation (HD) was applied. The average weight of the animals during the
final study was 2011 ± 297 gm. In each study, a sufficient amount of the cream to cover the
whole vaginal area, approximately 1 gm (approximately 2 to 3 ml), was used. Ten rabbits were
5 studied longitudinally for 3 weeks using control, low and high doses as defined above.

Dynamic MRI experiments were carried out on all of the rabbits when they were 5, 6 and
7 weeks old. All experiments were performed on a GE 1.5T Signa Scanner using the GE linear
extremity head coil. After the rabbit was anesthetized using a 25 mg/kg dose of Ketamine, a 25
gauge butterfly needle was placed into the ear vein for the injection of the contrast agent, and then
10 the rabbit was placed into the coil in a prone position. A fast spin echo (FSE) pulse sequence
(TR=3s, TE=112ms, 8 echo train) was applied to acquire the T2-weighted images as localizer and
anatomic images. A spin echo (SE) pulse sequence was applied to acquire the dynamic T1-
weighted images before and after injection of Gd-DTPA (gadolinium diethylenetriamine
pentaacetic acid) (0.1 mmol/kg, Magnevis, Berlex Lab. Inc.) as a short bolus. The dynamic study
15 started 15 min after the liposomal PGE-1 preparation was applied.

The alternations in blood flow in response to the use of liposomal PGE-1 were monitored
using dynamic contrast enhanced MRI and compartmental modeling as generally described by Su
and Nalcioglu (Magn. Reson. Med. 32(6):714-24, 1994; Magn. Res. Med. 34:402-11, 1995;
Magn. Res. Med. 39:259-69, 1998; Magn. Res. Med. 36:868-77, 1996, the disclosures of which
20 are incorporated herein by reference). This technique yields spatially resolved information
regarding vascular volume and tissue permeability. The physiologic parameters are obtained by
generating signal intensity time course curves for each pixel and subtracting the pre-contrast
baseline values from each point, resulting in a signal enhancement curve for each pixel. The
compartmental model is then used to derive vascular volume and tissue permeability by using a
25 nonlinear least squares fit through the signal enhancement vs. time curve. The details of the
method are described in the above references by Su and Nalcioglu.

In the present study the signal enhancement at 3 minutes post contrast agent injection was
used as a measure of blood flow through the tissue under consideration. Two regions of interest
(ROI) covering the vagina (VG) and leg muscle (MS) as a reference were used in the analysis.
30 In order to eliminate any systemic response we divided the signal enhancement in vagina by that
of the leg muscle (VG/MS). This provided us with a relative enhancement, or blood flow, of the
vagina compared to muscle. This value was calculated for each rabbit and for all 3 doses. The
relative blood flow enhancement, VG/MS, was then averaged over 10 rabbits for each dose.

MR images were analyzed, and they demonstrated that the blood flow was higher with the
35 low dose preparation than with the control, which did not contain any PGE-1. The results are
shown in Figure 5. Vaginal blood flow with the low dose preparation was higher than the control
with a statistical significance of $p < 0.01$, and vaginal blood flow with the high dose preparation

was higher than the control with a statistical significance of $p < 0.005$. Application of liposomal PGE-1 into the vagina caused a 45.9% increase in vaginal blood flow with a preparation containing 0.25 mg liposomal PGE-1, and a 59.5% increase with a preparation containing 2.0 mg liposomal PGE-1. The blood flow difference between the low dose preparation and low dose preparation was not statistically significant. Additionally, it was observed that the blood flow in the leg did not change significantly when the preparation containing PGE-1 was applied to the vagina. Table 5 shows the average blood flow in the leg muscle at each time point for the entire group of 10 rabbits. Table 6 shows the average relative blood flow in the vagina with respect to the leg muscle under the same conditions. The first row gives the relative average vaginal blood flow with respect to muscular blood flow at baseline with KY Jelly alone, at week 1 with a low dose preparation, and finally at week 2 with a high dose preparation. The second row gives the standard error in each measurement.

Table 5 - Blood flow in the Leg Muscle

	Control	Low Dose	High Dose
MS - mean	32.15	28.87	33.62
MS - SEM	1.11	1.53	2.95

**Table 6 - Relative Blood Flow in the Vagina
Normalized by the Blood Flow in the Leg Muscle**

	Control	Low Dose	High Dose
VG/MS - mean	2.07	3.02	3.02
VG/MS - SEM	0.11	0.29	0.18

The preceding description has been presented with reference to presently preferred embodiments of the invention. Workers skilled in the art and technology to which this invention pertains will appreciate that alterations and changes in the described structure may be practiced without meaningfully departing from the principal, spirit and scope of this invention. Accordingly, the foregoing description should not be read as pertaining only to the precise methods described, but rather should be read consistent with and as support to the following claims which are to have their fullest and fair scope.

1 CLAIMS:

5 1. A method for treating female sexual dysfunction or for female sexual enhancement comprising administering to the vagina a composition comprising a prostanoid compound having a vasodilating effect in a pharmaceutically acceptable vehicle, wherein the amount of prostanoid compound administered ranges from about 0.05 mg to about 3 mg.

10 2. The method according to claim 1, wherein the amount of prostanoid compound administered ranges from about 0.05 mg to about 2 mg.

3. The method according to claim 1, wherein the amount of prostanoid compound administered ranges from about 0.125 mg to about 1 mg.

15 4. The method according to claim 1, wherein the prostanoid compound is lyophilized.

5. The method according to claim 1, wherein the prostanoid compound is encapsulated within liposomes.

20 6. The method according to claim 5, wherein the liposomes are lyophilized.

7. The method according to claim 1, wherein the pharmaceutically acceptable vehicle is methylcellulose.

25 8. The method according to claim 1, wherein the composition is administered intravaginally.

9. The method according to claim 1, wherein the composition is administered extravaginally.

30 10. The method according to claim 1, wherein the prostanoid compound is prostaglandin E-1.

35 11. The method according to claim 10, wherein the amount of prostanoid compound administered ranges from about 0.05 mg to about 2 mg.

1 12. The method according to claim 10, wherein the amount of prostanoid compound administered ranges from about 0.125 mg to about 1 mg.

5 13. The method according to claim 10, wherein the prostanoid compound is encapsulated within liposomes.

 14. The method according to claim 13, wherein the liposomes are lyophilized.

10 15. The method according to claim 10, wherein the pharmaceutically acceptable vehicle is methylcellulose.

 16. The method according to claim 1, wherein the composition further comprises a surfactant capable of enhancing the bioavailability of the prostanoid compound.

15 17. A method for treating female sexual dysfunction or for female sexual enhancement comprising administering to the vagina a composition comprising lyophilized liposomal prostaglandin E-1 in methylcellulose, wherein the amount of prostaglandin E-1 administered ranges from about 0.125 mg to about 1 mg.

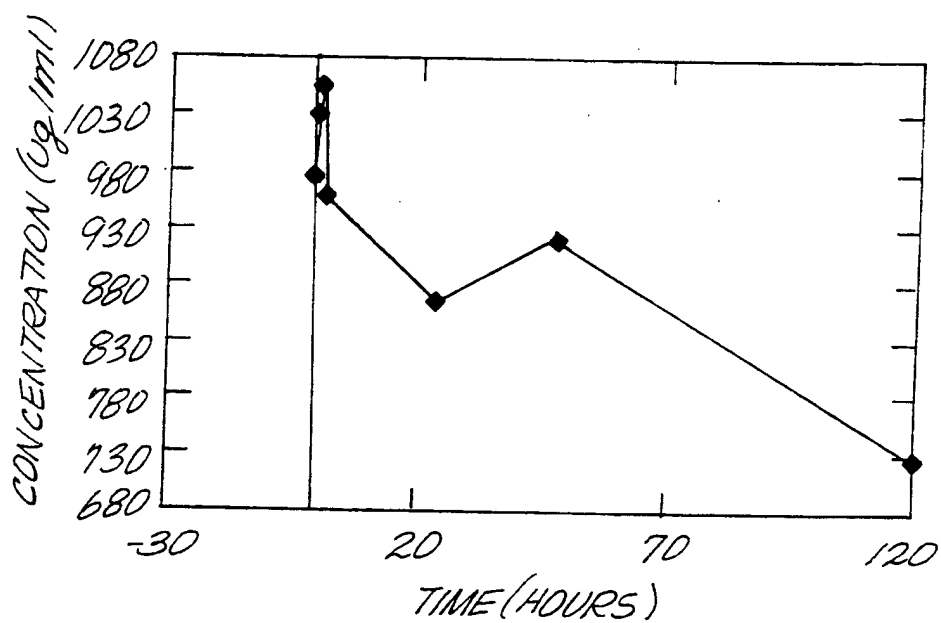
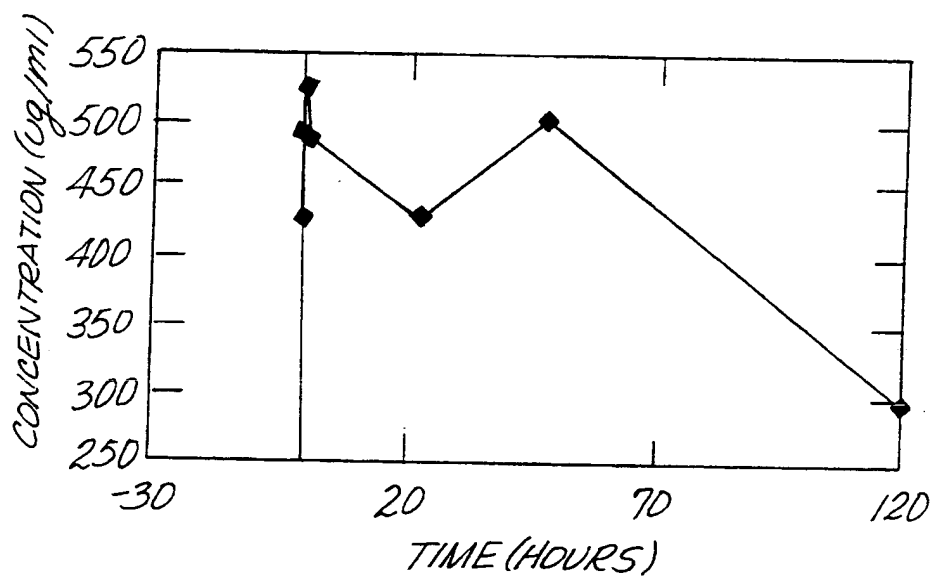
20 18. A method for enhancing blood flow in the vagina of a patient comprising administering to the vagina a composition comprising a prostanoid compound having a vasodilating effect in a pharmaceutically acceptable vehicle, wherein the amount of prostanoid compound administered ranges from about 0.05 mg to about 3 mg.

25 19. The method according to claim 18, wherein the amount of prostanoid compound administered ranges from about 0.05 mg to about 2 mg.

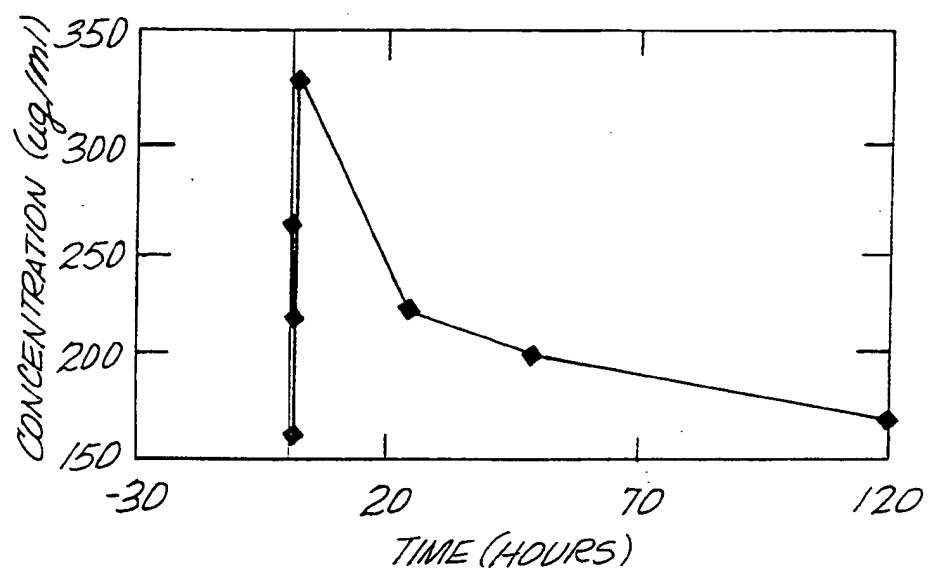
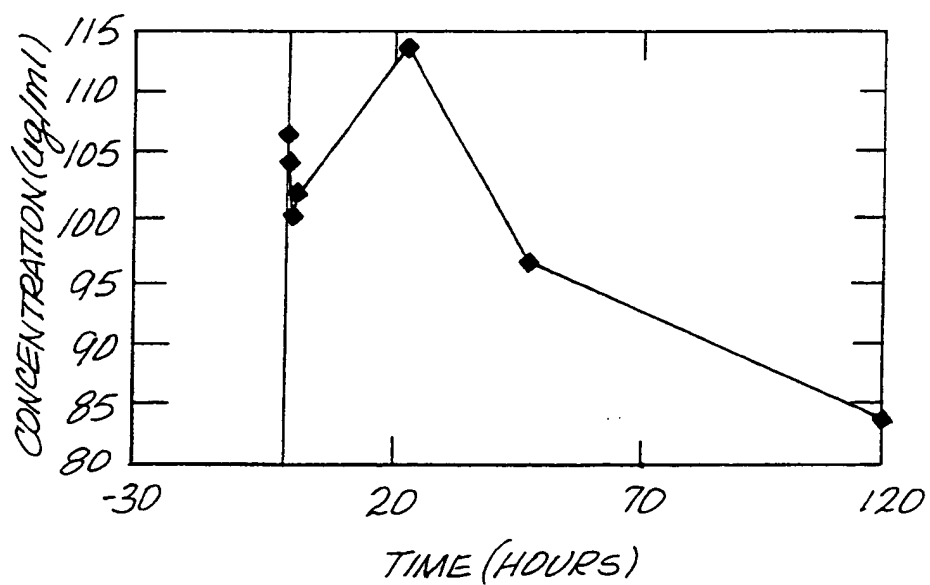
30 20. The method according to claim 18, wherein the amount of prostanoid compound administered ranges from about 0.125 mg to about 1 mg.

 21. The method according to claim 18, wherein the prostanoid compound is prostaglandin E-1.

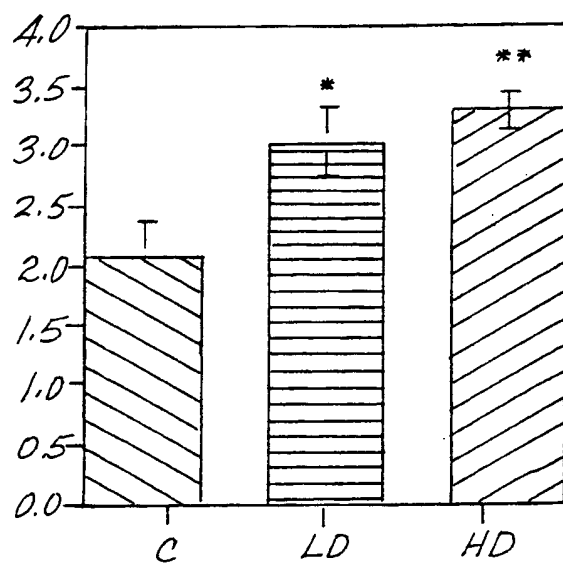
1/3

*Fig. 1**Fig. 2*

2/3

*Fig. 3**Fig. 4*

3/3

*Fig. 5*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09505

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/20

US CL :514/559

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/559

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, USPATFULL, HCAPLUS- treatment of sexual dysfunction, esp. in females employing prostanoid compounds herein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,576,290 A (HADLEY) 19 November 1996, col. 2, line 57 bridging col. 3, line 36.	1-21
Y	Database MEDLINE on STN, US National Library of Medicine, (Bethesda, MD, USA), AN 95174112, AKKUS, E. et al., 'Duplex ultrasonography after prostaglandin E1 injection of the clitoris in a case of hyperreactio luteinalis,' abstract, Journal of Urology, April 1995, 153(4), pages 1237-1238.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

04 JULY 1999

Date of mailing of the international search report

30 AUG 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

M. MOEZIE

Telephone No. (703) 308-1235